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Distinct and Overlapping Sarcoma Subtypes Initiated from Muscle Stem and Progenitor Cells

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Abstract

SUMMARY—Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children, while undifferentiated pleomorphic sarcoma (UPS) is one of the most common soft tissue sarcomas diagnosed in adults. To investigate the myogenic cell(s) of origin of these sarcomas, we used Pax7-CreER and MyoD-CreER mice to transform $Pax7^+$ and $MyoD^+$ myogenic progenitors by expressing oncogenic $Kras^{G12D}$ and deleting p53 *in vivo*. Pax7-CreER mice developed RMS and UPS, while MyoD-CreER mice developed UPS. Using gene set enrichment analysis, RMS and UPS each clustered specifically within their human counterparts. These results suggest that

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RMS and UPS have distinct and overlapping cells of origin within the muscle lineage. Taken together, we have established novel mouse models of soft tissue sarcoma from muscle stem and progenitor cells.

SIGNIFICANCE—Although muscle stem cells have been presumed to be a cell of origin for RMS, studies with constitutive Cre drivers expressed in Myf6-expressing cells or adipocyte P2-expressing cells suggest that cells of origin for RMS can be differentiated myofibers or adipogenic precursors, respectively. However, recent studies have demonstrated that Myf6 is expressed in muscle stem cell precursors, revealing a potential limitation of utilizing constitutive Cre drivers for cell of origin studies. Here, using inducible CreER mice, we mutate genes relevant to human RMS specifically in Pax7-expressing or MyoD-expressing cells. Our results suggest that RMS can be initiated in muscle stem cells, while UPS can be initiated in activated ($Pax7^{+}MyoD^{+}$) satellite cells.

INTRODUCTION

Soft tissue sarcomas account for approximately 10% of all newly diagnosed cancers in children, adolescents, and young adults; more than half of these tumors are rhabdomyosarcoma (RMS) (Arndt and Crist, 1999; Meyer and Spunt, 2004). RMS is characterized by the presence of rhabdomyoblasts and immunohistochemical (IHC) staining for myogenic transcription factors such as MyoD and Myogenin (Morotti et al., 2006). While patients with RMS who present with local disease are frequently cured, 5-year survival rates for children with metastatic disease are less than 25% (Arndt and Crist, 1999). By contrast, undifferentiated pleomorphic sarcoma (UPS) represents one of the most common histological subtypes of soft tissue sarcoma diagnosed in adults. Characterized by a lack of tissue specific differentiation, it is a diagnosis of exclusion. However, with careful examination, a subset of these tumors have been shown to express myogenic markers (Fletcher et al., 2001). Therefore, UPS may have multiple cells of origin with a convergent undifferentiated histological endpoint. Because approximately one-third of patients with RMS and UPS die from their disease, new therapies are needed for these cancers. Moreover, because contemporary treatment for these sarcomas often includes radiation and/or chemotherapy, survivors are at risk for second malignancies and other late effects of treatment. Thus, more targeted treatments for RMS and UPS with fewer side effects are needed.

Although the cell(s) of origin for RMS and UPS are not yet fully defined, candidates include the numerous myogenic cell types that are present in normal muscle tissue (Hettmer and Wagers, 2010; Linardic et al., 2005; Rubin et al., 2011). These unique myogenic cell populations can be identified by the expression of specific transcription factors. For example, satellite cells, which include the muscle stem cells, express Pax7 (Kuang et al., 2006). The satellite cell population is heterogeneous, consisting of quiescent $Pax7^{+}MyoD^{-}$ stem cells and another subpopulation that expresses MyoD (Buckingham and Relaix, 2007; Sacco et al., 2008). These two subpopulations have different capacities for self-renewal, underscoring functional heterogeneity within the satellite cell compartment. Upon injury or exercise, satellite cells become activated, express Pax7 and MyoD, and differentiate into myoblasts, which function as transit amplifying cells (Sabourin et al., 1999).

Because human eRMS frequently expresses both Pax7 and MyoD (Tiffin et al., 2003), it seems reasonable that a Pax7 or MyoD-expressing myogenic progenitor would be a candidate cell of origin in eRMS. However, a recent study reported that differentiating myoblasts that express the terminal differentiation marker Myf6 give rise to eRMS. Using *Myf6*-Cre;$Trp53^{Fl/Fl}$ mice, the tumor suppressor p53 was ablated in $Myf6^{+}$ cells throughout development and these mice preferentially gave rise to eRMS while a *Pax7*-

CreER;*Trp53*$^{Fl/Fl}$ mouse developed UPS (Rubin et al., 2011). Here, we used mice that express tamoxifen-inducible CreER from either the endogenous Pax7 or MyoD promoters to simultaneously drive expression of oncogenic Kras and delete *Trp53*. We found that Pax7^{+} satellite cells are cells of origin for RMS and UPS, while Pax7^{+}MyoD^{+} myogenic progenitors are cells of origin for UPS, which suggests that muscle stem cells are a cell of origin for RMS.

RESULTS

Transformation of Pax7^{+} myogenic progenitors *in vivo* gives rise to a spectrum of myogenic sarcomas

We have previously utilized genetically engineered mice carrying conditional oncogenic *Kras*^{G12D} and *Trp53* knockout alleles (*LSL-Kras*$^{G12D/+}$;*Trp53*$^{Fl/Fl}$) to generate a spatially- and temporally- restricted mouse model of soft tissue sarcoma (Kirsch et al., 2007). To restrict mutations of Kras and p53 to Pax7^{+} myogenic progenitors, we crossed Pax7-CreER^{T2} mice (Lepper et al., 2009) into the *LSL-Kras*$^{G12D/+}$;*Trp53*$^{Fl/Fl}$ model to generate ***P****ax7*$^{CE/+}$;*LSL-**K**ras*$^{G12D/+}$;*Tr**p**53*$^{Fl/Fl}$ mice, hereafter termed P7KP. We injected greater than 6 week-old P7KP mice (n=17) with systemic tamoxifen via intraperitoneal (IP) delivery. The mice developed multiple tumors with 100% penetrance within one to two months at various anatomic locations including clinically relevant sites such as the orbit (Figure 1A). Tumors (n=67, 3.9 tumors per mouse on average) displayed a histological spectrum ranging from UPS to RMS and included sarcomas that mimicked embryonal RMS (eRMS), pleomorphic RMS (pRMS), myogenic UPS, and non-myogenic UPS (Figures 1B-1I; Figures S1A-S1N). In terms of anatomic distribution, 37% of sarcomas appeared in the body wall, 31% in the extremities, 23% in the head and neck, and 9% were subcutaneous (Figure 1J). Based on hematoxylin and eosin (H&E) stained histological sections, 62% of the tumors were UPS with the remainder being RMS, which were predominantly eRMS-like, though some tumors had pRMS-like or rarely aRMS-like features (Figure 1K). Although 100% of the eRMS and 89% of the pRMS were classified as myogenic by staining for MyoD, Pax7, or Myogenin, 74% of the UPS stained for at least one of these myogenic markers (Figure 1L). Therefore, transformation of Pax7^{+} myogenic progenitors *in vivo* gives rise to a spectrum of sarcomas ranging from RMS to UPS.

Generation and characterization of *MyoD*$^{CE/+}$ mice

Given the spectrum of sarcomas that arose in the P7KP mice, we hypothesized that different subpopulations of Pax7^{+} cells give rise to RMS and UPS because Pax7 is expressed in quiescent satellite cells, activated satellite cells, and myoblasts. Because MyoD is expressed in activated satellite cells and myoblasts, we next studied sarcoma development in mice in which *CreER* is driven by MyoD. First, we generated *MyoD*$^{CE/+}$ mice in which CreER was knocked into the *MyoD* locus and expressed from the endogenous *MyoD* promoter (Figure 2A; Figures S2A-S2F). To compare the expression of Cre in *MyoD*$^{CE/+}$ and *Pax7*$^{CE/+}$ mice, we crossed these strains to the dual reporter mouse *R26*^{mTmG} (Muzumdar et al., 2007). The *R26*^{mTmG} reporter expresses membrane targeted tandem-Tomato in the absence of Cre. After Cre-mediated recombination, the tandem-Tomato gene is deleted and membrane targeted GFP is expressed constitutively in the cell and its descendants. We injected *Pax7*$^{CE/+}$;*R26*$^{mTmG/+}$ and *MyoD*$^{CE/+}$;*R26*$^{mTmG/+}$ mice (greater than 6 weeks of age) with IP tamoxifen to determine if there was a difference in membrane GFP (and thus Cre) expression in skeletal muscle cells by flow cytometry. We observed that the *Pax7*$^{CE/+}$;*R26*$^{mTmG/+}$ muscle contained significantly more GFP^{+} cells than the *MyoD*$^{CE/+}$;*R26*$^{mTmG/+}$ muscle (Figure 2B). This result is consistent with Pax7 expression in adult muscle satellite cells (Kuang et al., 2006; Lepper et al., 2009; Sacco et al., 2008),

because MyoD expression only becomes prominent in satellite cells after activation (Cornelison et al., 2000; Zammit et al., 2002).

To further explore expression of CreER in the *MyoD*$^{CE/+}$ mice, we isolated myoblasts from *MyoD*$^{CE/+}$ mice and co-cultured them with fibroblasts shedding RCAS-GFP virus for 48 hours. The *CreER* knock-in allele at the MyoD promoter contains the avian retroviral receptor *tva*, which is expressed downstream from an internal ribosomal entry site (*IRES*) on the same mRNA as the *CreER* (Figure S2A). Therefore, cells that express CreER from the *MyoD* promoter will also express tva and will thereby be susceptible to RCAS-GFP infection. Figure S2F shows that because myoblasts from *MyoD*$^{CE/+}$ mice can be successfully infected with RCAS-GFP, they express the *CreER-IRES-tva* transcript from the *MyoD* promoter (Figure S2F). Additionally, myoblasts isolated from *MyoD*$^{CE/CE}$ mice co-express MyoD and CreER at passage 1 (Figure S2G).

To ascertain if *MyoD*$^{CE/+}$ mice drive tamoxifen-inducible Cre activity in differentiating myogenic progenitors *in vivo*, we injured the tibialis anterior (TA) muscles of *Pax7*$^{CE/+}$;*R26*$^{mTmG/+}$ and *MyoD*$^{CE/+}$;*R26*$^{mTmG/mTmG}$ mice to induce satellite cell activation (Sacco et al., 2008). The TAs were injured with a single intramuscular (IM) injection of cardiotoxin on day 0. Then, the mice were injected with 5 daily doses of IP tamoxifen beginning on day 1 (Figure 2C). The mice were euthanized on day 6 and the skeletal muscle was collected for sectioning. We observed that similar to *Pax7*$^{CE/+}$;*R26*$^{mTmG/+}$ mice, the *MyoD*$^{CE/+}$;*R26*$^{mTmG/mTmG}$ mice had GFP^{+} regenerating myofibers at the site of injury (Figure 2C). Taken together, these results indicate that CreER in *MyoD*$^{CE/+}$ mice is expressed in activated MyoD^{+} myogenic progenitors during muscle regeneration and/or repair.

Transformed MyoD^{+} myogenic progenitors give rise to UPS

To assess the spectrum of sarcomas generated from MyoD^{+} cells, we crossed the *MyoD*$^{CE/+}$ mice to *LSL-Kras*$^{G12D/+}$;*Trp53*$^{Fl/Fl}$ mice to generate ***M****yo****D***$^{CE/+}$; *LSL-****K****ras*$^{G12D/+}$;*Tr****p****53*$^{Fl/Fl}$ mice, hereafter referred to as MDKP mice. We injected greater than 6 week-old MDKP mice with IP tamoxifen (Figure 3A). Like their P7KP counterparts, MDKP mice developed tumors (n=12) at a variety of clinically relevant anatomic sites. Interestingly, MDKP mice developed tumors with increased latency with a median tumor-free survival of 153 days compared to P7KP mice, which developed tumors at a median time of 44 days (Figure 1A vs. Figure 3A). In contrast to the P7KP mice, which often developed multiple sarcomas after IP tamoxifen, the MDKP mice usually developed a single sarcoma after IP tamoxifen. The increased latency and decreased number of tumors per mouse is likely due to the small number of cells expressing MyoD in the MDKP mice under homeostatic conditions, as the mice were injected at an age when there are few MyoD-expressing cells (Chakkalakal et al., 2012; Lee et al., 2012).

Sarcomas that developed in the MDKP mice were exclusively UPS (n=12). Similar to the UPS in P7KP mice, the UPS in MDKP mice could be subdivided into myogenic UPS (Figures 3B & 3C; Figures S3F & S3G) and non-myogenic UPS (Figures 3D & 3E; Figures S3A – S3E). The anatomic distribution of the tumors derived from the MDKP mice was similar to the tumors in the PK7P mice and included the extremities, body wall, and the head and neck region (Figure 3F). While the histological distribution of sarcomas in the MDKP model was restricted to UPS (Figure 3G), 60% stained with either MyoD or Myogenin (Figure 3H). These results indicate that MyoD^{+} myogenic progenitors are a cell of origin for both myogenic and non-myogenic UPS.

P7KP and MDKP derived UPS cluster separately from P7KP derived RMS at the gene expression level and mimic their human counterparts by GSEA

To investigate if the histological spectrum of tumors from P7KP and MDKP mice reflects underlying molecular differences, we isolated mRNA from sarcomas derived from MDKP mice and all 3 histological variants (UPS, pRMS, and eRMS) from P7KP mice. We first compared the gene expression profiles by principal component analysis (PCA). This unbiased approach transforms the many correlated gene expression measurements for each sample into a set of uncorrelated principal component scores, while still capturing the greatest possible variation in gene expression. We plotted all of the samples on a two-dimensional plot using the principal component scores from the first two principal components (Figure 4A). Samples that are proximal on the plot have a more similar gene expression profile than samples that are distal from one another. When examining the first principal component scores, we found that the P7KP derived eRMS-like tumors clustered separately from MDKP and P7KP derived UPS-like tumors (Figure 4A). As expected, MDKP derived tumors, which appeared to be UPS by histology, clustered more closely with P7KP derived UPS-like tumors than the eRMS-like tumors when using the first principal component scores. P7KP derived pRMS-like tumors did not cluster together using the first principal component scores and were distributed evenly throughout the clusters of other subtypes, suggesting that they do not represent a distinct subtype at the molecular level.

To further analyze the gene expression data, we performed a second unsupervised approach using hierarchical clustering. In good agreement with the PCA results, hierarchical clustering demonstrated that MDKP derived tumors tend to cluster more closely with P7KP derived UPS-like tumors (UPS-like clade) compared to P7KP derived eRMS-like and pRMS-like tumors, which tended to cluster into a separate group (RMS-like clade) (Figure 4B). The two UPS tumors that clustered within the RMS-like clade stained positively for either MyoD or Myogenin. These data further suggest that there are underlying molecular differences between different histological subtypes of mouse sarcomas.

To investigate whether the mouse sarcoma subtypes are similar to specific human sarcomas at the gene expression level, we performed gene set enrichment analysis (GSEA). GSEA is an established method of measuring the simultaneous differential expression of multiple genes in a gene set between two classes (i.e. tumor types A and B). We defined a gene set comprised of the genes most significantly ($p< 0.0001$) upregulated in P7KP RMS (eRMS and pRMS) compared to the UPS derived from the MDKP mice (Figure 4C; Figure S4A). Then, we used GSEA to test whether this gene set upregulated in the P7KP RMS tumors could be used to distinguish between human RMS and other human soft tissue sarcomas. Using a previously annotated human sarcoma gene expression dataset (Baird et al., 2005), we performed GSEA for each type of sarcoma by comparing each individual sarcoma subtype to all other sarcomas in the dataset. In good agreement with the histological data, when the gene set comprised of genes upregulated in the P7KP RMS was applied to a human dataset of multiple soft tissue sarcoma subtypes, the P7KP derived mouse RMS enriched in human RMS, validating this system as a model for human RMS (Figure 4C, $p=0.002$, NES=1.88, FDR=0.024). To determine whether the P7KP UPS and MDKP UPS sarcomas mimic human sarcoma at the molecular level, we also generated a gene set comprised of the genes most significantly ($p< 0.001$) upregulated in the mouse UPS from P7KP and MDKP mice compared to the mouse RMS from P7KP mice (Figure 4C; Figure S4B). This gene set was then applied to the human sarcoma dataset. Like the gene set from P7KP RMS sarcomas, the gene set from mouse UPS derived from P7KP and MDKP mice enriched in its human counterpart, validating the mouse UPS as models of human UPS (Figure 4C, $p<0.001$, NES=1.85, FDR<0.012).

DISCUSSION

We used the CreER-loxP system to express oncogenic Kras and delete p53 specifically in Pax7-expressing or MyoD-expressing cells to show that both $Pax7^+$ and $MyoD^+$ myogenic cells are cells of origin for both myogenic and nonmyogenic soft tissue sarcomas. Sarcomas generated by $Pax7^+$ myogenic cells displayed histological diversity, ranging from Undifferentiated Pleomorphic Sarcoma (UPS) to differentiated eRMS, whereas $MyoD^+$ cells gave rise to a more restricted UPS phenotype. Furthermore, gene sets generated by comparing mouse UPS and mouse RMS distinguished human UPS and RMS from other soft tissue sarcomas. Therefore, these mouse sarcomas not only appear histologically similar to human RMS and UPS, but GSEA suggests that they phenocopy these tumors at the molecular level as well.

We used inducible Cre (CreER) alleles for cell of origin studies of sarcomas to temporally activate gene mutations after development. Although conditional gene manipulation using a constitutive Cre allele is a powerful technique to activate or delete genes *in vivo* to model cancer, unanticipated expression patterns of Cre recombinase throughout development can present challenges for pinpointing cells of origin for cancer (Heffner et al., 2012; Lee et al., 2013; Sambasivan et al., 2013). For example, Rubin et al. (2011) used Myf6-Cre mice to conclude that eRMS can develop from differentiating Myf6-expressing myoblasts, but it has recently been shown that Myf6 is not only expressed in differentiating myoblasts and myotubes (Rubin et al., 2011), but is also expressed in satellite cell precursors during development (Sambasivan et al., 2013). Therefore, the cell of origin for sarcomas from Myf6-Cre mice cannot be defined unambiguously. Here, we used tamoxifen-activated Cre (CreER) alleles, which can be activated after development, to show that mutant Kras, in concert with loss of *Trp53*, initiates eRMS and UPS in satellite cells, while UPS develops from MyoD expressing cells.

By comparing the sarcomas that developed in the P7KP and MDKP mice to each other, we find that $Pax7^+$ and $MyoD^+$ progenitor cells can give rise to distinct and overlapping myogenic soft tissue sarcomas *in vivo*. Although P7KP mice generate RMS and UPS after systemic tamoxifen administration, systemic tamoxifen into MDKP mice preferentially generates UPS. Consistent with this result, microarray analysis suggests that UPS tumors from MDKP mice preferentially cluster with P7KP derived UPS, while P7KP derived eRMS clustered in a separate class altogether. These data imply that the transcriptional profiles of UPS derived from P7KP and MDKP mice are more similar to each other than they are to RMS (eRMS and pRMS) generated in the P7KP mice; in addition, only the Pax7-expressing cells give rise to a spectrum of RMS, suggesting that $Pax7^+MyoD^+$ cells are a cell of origin for UPS *in vivo*. These data also raise the possibility that transformation of a myogenic progenitor cell (not the muscle stem cell) gives rise to a more undifferentiated sarcoma.

There are several salient features of these myogenic sarcoma models. First, the sarcomas are initiated by mutations in genes that are relevant to human RMS and UPS. *TRP53*, which is mutated in 16.7% of human UPS (Barretina et al., 2010) and has been reported to be mutated in human RMS (Felix et al., 1992; Merlino and Helman, 1999; Mulligan et al., 1990; Tsumura et al., 2006) is deleted in this model. Mutant *RAS*, primarily *KRAS* and *NRAS*, is also observed in human eRMS (Martinelli et al., 2009; Stratton et al., 1989). In this mouse model, oncogenic Kras is expressed from its endogenous promoter at physiological levels (Tuveson et al., 2004). Second, tamoxifen activates $Kras^{G12D}$ and *Trp53* conditional mutations in an inducible fashion via CreER in postnatal $Pax7^{CE/+}$ and $MyoD^{CE/+}$ mice. Therefore, the initiating mutations are restricted to either $Pax7^+$ (Lepper et al., 2009) or $MyoD^+$ cells (Figures 2 and S2), so that the consequences of transforming these cells can be studied *in vivo*. Our results do not exclude more differentiated cells, such as myotubes and

myofibers, as potential cells of origin for myogenic sarcomas. It is possible that initiating mutations in myotubes or myofibers will also give rise to myogenic sarcomas. This latter possibility will be explored in future studies using both Myogenin-CreER and Myf6-CreER mice.

In summary, we determined that $Pax7^+$ myogenic progenitors are a cell of origin for both UPS and RMS. We also found that $MyoD^+$ myogenic progenitors are a cell of origin for UPS. Taken together, these results suggest that $Pax7^+MyoD^-$ quiescent satellite cells can be a cell of origin for RMS and that $Pax7^+MyoD^+$ cells can be a cell of origin for UPS, at least in the context of *Kras* and *Trp53* mutations. Finally, the two novel genetically engineered mouse models for UPS and RMS reported here may be useful for testing novel therapies for RMS and UPS in the pre-clinical setting.

EXPERIMENTAL PROCEDURES

Mice and sarcoma generation

Mice were on a mixed 129 S_4/SvJae and C57/Bl6 background. *LSL-Kras*^{G12D} mice (Tuveson et al., 2004) were provided by T. Jacks (Massachusetts Institute of Technology). *Trp53*^{Fl} mice (Meuwissen et al., 2003) were obtained from Anton Berns (The Netherlands Cancer Institute). The *Pax7*^{CE} mice have been previously described (Lepper et al., 2009), and the *MyoD*^{CE} mice were generated as described in the Supplemental Experimental Procedures. *R26*^{mTmG} reporter mice were developed by Liqun Luo and obtained from The Jackson Laboratory. Sarcomas were generated using intraperitoneal injections of tamoxifen (Sigma-Aldrich) dissolved in EtOH and diluted in corn oil (10μl 20mg/ml tamoxifen per gram body weight). All animal experiments were performed according to protocols approved by the Duke University Institutional Animal Care and Use Committee.

Immunofluorescence and Immunohistochemistry

Tumor specimens were fixed in 10% formalin/70% EtOH and paraffin embedded. 4μm sections were stained with H&E or with antibodies (see Supplemental Experimental Procedures). All IHC was performed using Vectastain Elite ABC Kit (Mouse IgG) and 3,3′-Diaminobenzidine tetrahydrochloride (Sigma-Aldrich). Immunofluorescence and immunohistochemistry images were captured on a Leica DM5500B microscope using Leica Application Suite software.

Microarray Processing and Analysis

Gene expression of RMS and UPS was determined using Affymetrix 430A 2.0 arrays (Affymetrix). RNA was isolated using the RNeasy Fibrous Tissue Mini Kit (Qiagen). The human dataset was downloaded from GEO (GSE2553) (Baird et al., 2005). All microarray data have been deposited in GEO (GSE46836). See Supplemental Experimental Procedures for further details.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

1. Mouse RMS (mRMS) can be initiated in $Pax7^{+}MyoD^{-}$ muscle stem cells.
2. Mouse UPS (mUPS) can be initiated in $Pax7^{+}MyoD^{+}$ myogenic progenitors.
3. mUPS cluster separately from mRMS by PCA and hierarchical clustering.
4. mUPS and mRMS enrich in their human counterparts by GSEA.

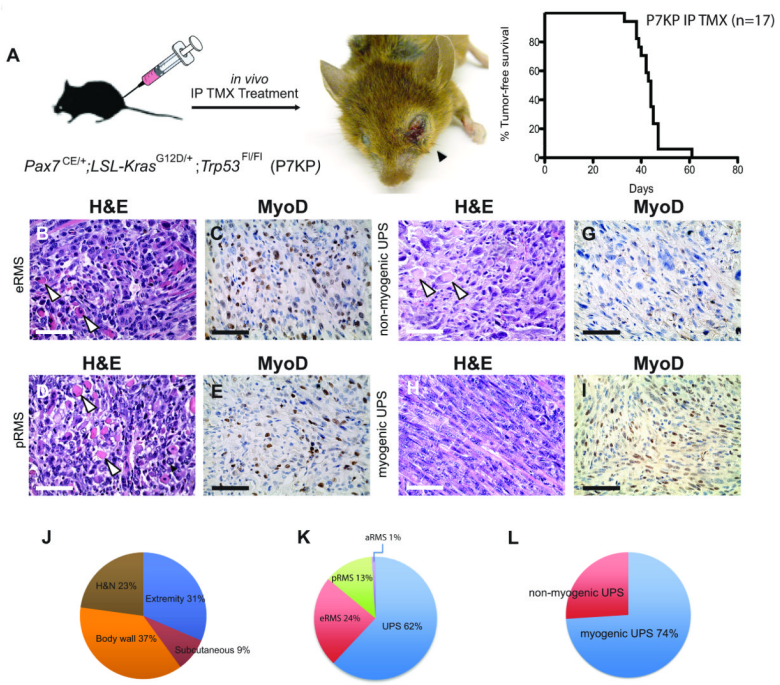


Figure 1. Transforming Pax7^{+} myogenic progenitors *in vivo* generates RMS and UPS

(A) P7KP mice (n=17) were injected with systemic tamoxifen (IP TMX) and generated tumors (n=67) at clinically relevant anatomic locations, such as the orbit. Median tumor free survival was 44 days. **(B)** eRMS displayed small round, blue cell histology with rhabdomyoblasts (arrowheads) by H&E, and were positive for the myogenic marker MyoD **(C)**. **(D)** pRMS displayed characteristic large rhabdomyoblasts (arrowheads) with spindle cell histology by H&E, and were positive for the myogenic marker MyoD **(E)**. **(F)** Non-myogenic UPS displayed characteristic spindle cell histology with giant cells (arrowheads) by H&E, but lacked MyoD expression **(G)**. **(H)** In contrast, myogenic UPS displayed spindle cell histology, but were positive for MyoD **(I)** (40X, scale bar=50μm). **(J)** Anatomic distribution of sarcomas generated in P7KP mice injected with IP tamoxifen. **(K)** Histological distribution of sarcoma subtypes in P7KP mice injected with IP tamoxifen. **(L)** Myogenic distribution in the P7KP derived UPS sarcoma subset. See also Figure S1.

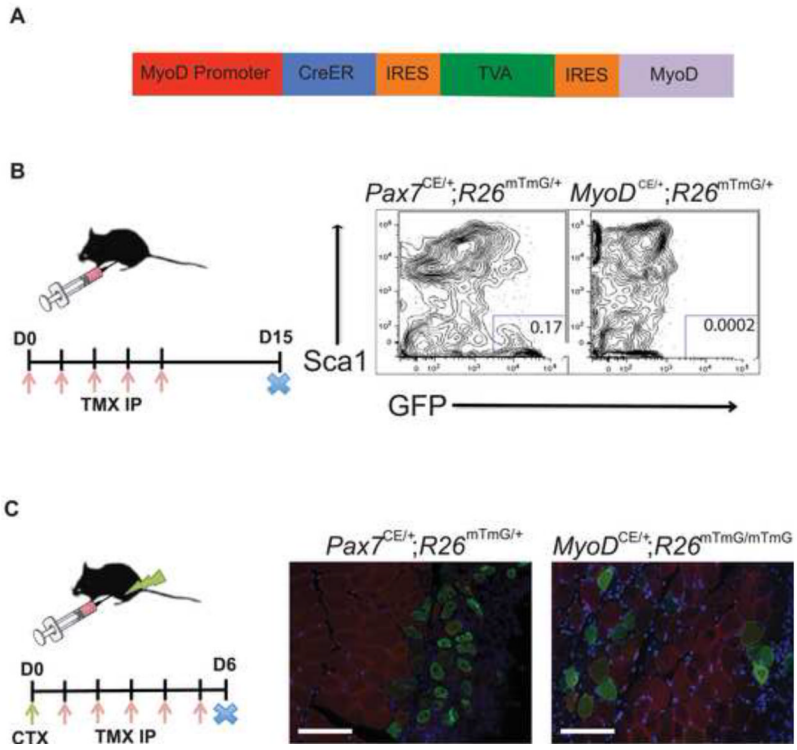


Figure 2. Characterization of *MyoD*$^{CE/+}$ mice

(A) Schematic of the *MyoD*$^{CE/+}$ allele. CreER was targeted to the endogenous *MyoD* locus. In addition, the avian tumor virus receptor A (tva) and MyoD are expressed from dual Internal Ribosome Entry Sites (IRES). **(B)** *Pax7*$^{CE/+}$;*R26*$^{mTmG/+}$ (n=2) and *MyoD*$^{CE/+}$;*R26*$^{mTmG/+}$ (n=2) mice were injected (IP) with tamoxifen (TMX) for five consecutive days and sacrificed ten days later. *Pax7*$^{CE/+}$;*R26*$^{mTmG/+}$ mice contained a GFP^{+} satellite cell population while *MyoD*$^{CE/+}$;*R26*$^{mTmG/+}$ mice had no identifiable GFP^{+} population. **(C)** Following injury with intramuscular cardiotoxin (CTX) and five daily TMX IP injections, both *Pax7*$^{CE/+}$;*R26*$^{mTmG/+}$ and *MyoD*$^{CE/+}$;*R26*$^{mTmG/mTmG}$ mice (n=3) exhibited GFP^{+} regenerating muscle fibers (40X, scale bar=50μm). See also Figure S2.

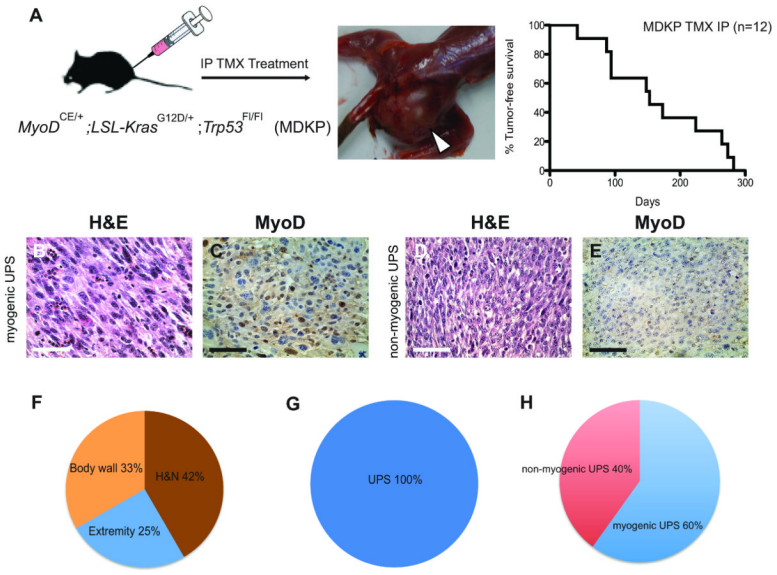
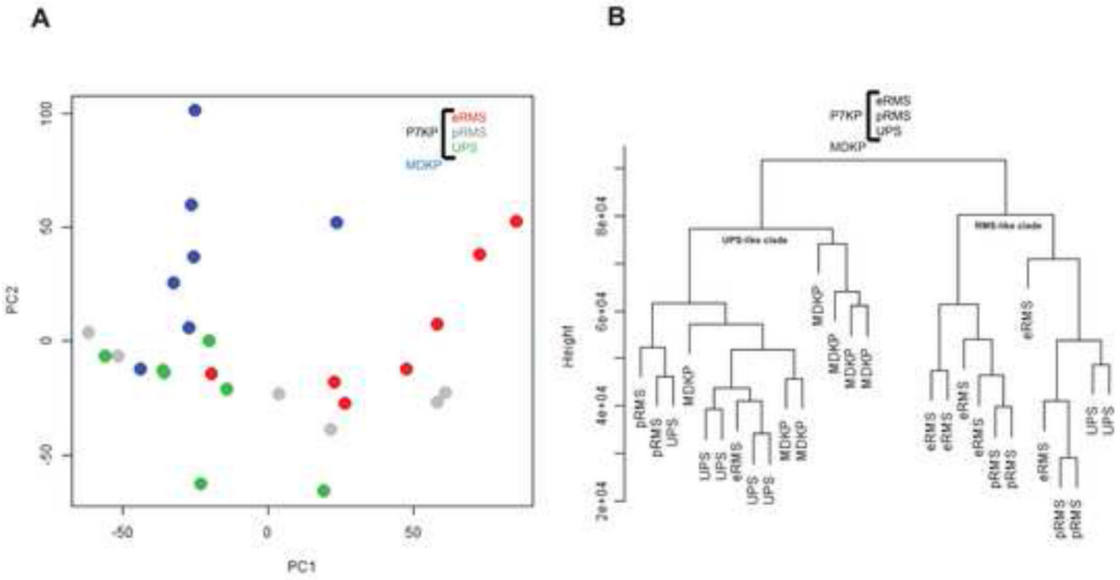


Figure 3. Transforming MyoD^{+} myogenic progenitors *in vivo* generates UPS

(A) MDKP mice injected with IP tamoxifen (TMX) generated single tumors at clinically relevant anatomic locations, such as the proximal thigh. Median tumor-free survival was 153 days (n=12 mice). **(B)** MDKP derived myogenic UPS had spindle cell histology by H&E and expressed the myogenic marker MyoD **(C)**. In contrast, non-myogenic UPS maintained the classic spindle cell and pleomorphic histology **(D)**, but lacked immunoreactivity for MyoD **(E)** (40X, scale bar=50μm), Pax7, and Myogenin (Figure S3). **(F)** Anatomic distribution of sarcomas generated in MDKP mice injected with IP tamoxifen. **(G)** Histological distribution of sarcomas generated in MDKP mice injected with IP tamoxifen. **(H)** Distribution of myogenic staining in MDKP derived UPS. See also Figure S3.



C

	P7KP RMS vs MDKP			P7KP UPS and MDKP vs P7KP RMS		
Tumor Type	NES	p-value	FDR	NES	p-value	FDR
Rhabdomyosarcoma (RMS)	**1.88**	**0.002**	**0.024**	-1.32	0.141	0.564
Malignant Fibrous Histiocytoma (UPS)	-0.75	0.794	0.827	**1.85**	**<0.001**	**<0.012**
Ewing's Sarcoma	-0.86	0.647	0.776	0.71	0.855	0.855
Liposarcoma	1.16	0.261	0.626	-0.76	0.806	0.855
Leiomyosarcoma	1.54	0.053	0.212	-1.09	0.342	0.777
Fibrosarcoma	0.72	0.827	0.827	1.01	0.453	0.777
Osteosarcoma	1.04	0.391	0.67	0.83	0.692	0.855
Synovial Cell Sarcoma	-1.56	0.029	0.174	-1.53	0.045	0.27
Gastrointestinal Stromal Tumor	-0.97	0.497	0.746	-0.93	0.549	0.824
Malignant Hemangiopericytoma	0.89	0.601	0.776	0.73	0.82	0.855
Dermato Tumor Type E	1.03	0.384	0.67	-1.02	0.444	0.777
Malignant Peripheral Nerve Sheath Tumor	1.33	0.126	0.378	-1.02	0.446	0.777

Figure 4. Sarcomas cluster by histologic subtype using unbiased genomic analysis

(A) Principal component analysis clustered P7KP derived eRMS separately from P7KP derived UPS and MDKP derived tumors along the first principal component (PC1). The second principal component (PC2) separated the UPS P7KP sarcomas from the MDKP sarcomas. **(B)** Unsupervised hierarchical clustering separated the sarcomas into an RMS-like clade that includes most P7KP derived eRMS and pRMS and a UPS-like clade that includes most P7KP derived UPS and MDKP derived tumors. **(C)** GSEA demonstrates that a gene set derived from P7KP RMS (eRMS and pRMS) enrich in human RMS, while a gene set from P7KP derived UPS and MDKP sarcomas enrich in human UPS. See also Figure S4.